REMARKS/ARGUMENTS

Claims 20-21, 24, 26, 28-31, and 35-38 are pending. No amendment is made to any of the pending claims. Reconsideration of the present application in view of the following remarks is respectfully solicited.

I. Obviousness rejection of claims 20-21, 24, 26, 28-31,35-36, and 38

The Examiner maintains the rejection of claims 20-21, 24, 26, 28-31, 35-36, and 38 as being unpatentable under 35 U.S.C. § 103(a) over Roth et al (U.S. Patent No. 5,545,535) in view of Akai et al. (U.S. Patent No. 5,891,731) and Yue ST (U.S. Patent No. 5,656,449). We disagree.

Independent claim 20 recites a method of preparing an assay sample for detecting bacteria by a flow cytometer. The method comprises:

"providing a **diluent comprising** a cationic surfactant, a buffer for maintaining a pH of 2.0-4.5 and an effective amount of a substance capable of reducing nitrite ions **and a staining solution comprising** a polymethine dye for staining bacteria;

mixing a urine sample with the diluent; and

preparing the assay sample by **mixing the mixture of the urine sample and the diluent with the staining solution**. .. ."

(Emphasis added.)

Another independent claims claim 38, which is directed to a method of staining bacteria, comprises the same steps as recited in claim 20. The remaining pending claims all depend from claim 20.

First, as explained in Applicants' previous response, none of the cited art discloses a step of providing a **diluent** as recited in the claims of the present application or **a step of mixing a urine sample with the diluent prior to the step of mixing the urine sample with a staining solution**. Indeed, none of the cited art provides any reason to do so. Unlike the present application, none of the cited art recognizes

the benefit of reducing the nitrite ions in the urine sample to improve the accuracy of the fluorescence assay.

In response, the Examiner repeatedly argues, "Claim 20 does not recite that 'mixing a urine sample with the diluent prior to the step of mixing the urine sample and the diluent with the staining solution.' According to claim 20, the diluent comprises the polymethine dye for staining bacteria." See, e.g., pages 8-9, item 10. This argument lacks merit.

A reasonable reading of independent claim 20 (or independent claim 38) would not lead to the Examiner's conclusion that the diluent comprises a dye for staining bacteria. Rather, the dye is present in the staining solution which is added to a mixture of urine sample and diluent. It is clear from claim 20 that the diluent and staining solution are two separate compositions. Specifically, the diluent comprises a cationic surfactant, a buffer for maintaining a pH of 2.0-4.5 and an effective amount of a substance capable of reducing nitrite ions. The staining solution comprises a polymethine dye for staining bacteria. Thus, in the presently claimed method the substance capable of reducing nitrite ions that is contained in the diluent is able to react with the nitrite ions in the urine before the staining solution is introduced thereby resulting in improved accuracy of the fluorescence assay.

Moreover, as described in independent claim 20 (or independent claim 38), the method comprises, among other things, "mixing a urine sample with the diluent' and "mixing the mixture of the urine sample and the diluent with the staining solution." (Emphasis added.) In other words, in accordance with claim 20, a urine sample should be first mixed with the diluent, and then the mixture so formed is mixed with the staining solution. It is abundantly clear from the language of claim 20 (or claim 38), the clamed process require "mixing a urine sample with the diluent prior to the step of mixing the urine sample and the diluent with the staining solution", even though such language is not recited in claim 20 (or claim 38) literally.

Therefore, for at least the reason discussed above alone, independent claims 20, 38, and the remaining dependent claims are all patentable in view of the cited art under 35 U.S.C. § 103(a).

If upon considering Applicants' above explanation, the Examiner still believes that the claims of the present application are not clear in distinguishing from the prior art, then to expedite the prosecution, Applicants are willing to consider any suggestion the Examiner may offer in amending the claims for better format and clarity. Should the Examiner have any suggestion, the Examiner is respectfully requested to contact the undersigned representative.

Second, in the previous response, Applicants explained that none of the cited art discloses a diluent comprising a buffer for maintaining a **pH of 2.0-4.5.** Rather, the cited art teaches away from the pH range recited in the claims of the present application. For example, Roth discloses several specific pH values, including 7.4, 10, and 8.2 (*see* col. 10, Table 1, footnotes 1 and 4, col. 36, line 39), which all depart significantly from the range recited in the claims of the present application. Akai discloses at col. 8, lines 23-37 that the suitable pH range is 6.0-11.0, preferably 7.0-11.0, and more preferably 8.0-9.5. According to Akai, "when the pH is lower than this range, erythocytes become fragile and hemolysis is apt to take place whereby accurate measurement of reticulocytes becomes difficult." Yue discloses at col. 6, lines 14, that the pH of the staining solution is typically between 6.5 and 8, which is significantly different from the range recited in the claims of the present application.

In response, the Examiner argues: "According to Akai et al., pH lower than 8.0-9.5 would lead to hemolysis of erythrocte and reticulocytes would be difficult [sic]. Erythocyte and reticulocytes are red blood cells, therefore one of ordinary skill in the art would be motivated to optimize the pH to detect bacteria in bodily fluid and distinguish them from other samples, found in urine and other bodily fluids, such as red blood cells. Therefore, the combined art does not teach away from the pH range recited in the claims of the present application. One of ordinary skill in the art would have been motivated to optimize the pH to detect

only the bacteria in the bodily fluids, and distinguish bacteria from other cells, such as erythocytes and reticulocytes." This argument lacks merit.

As the Examiner acknowledges, to assay a urine sample or other bodily fluids, one need to distinguish bacteria from other substances, such as erythocytes and reitculocytes. If one cannot detect or determine substances such as erythocytes and reitculocytes then s/he would not accurately distinguish bacteria from these other substances contained in the urine sample of other bodily fluids. Therefore, a person of ordinary skill in the art would not depart from the general scope of pH, i.e., 8.0-9.5, as taught in Akai or other cited art. In particular, this person would avoid using a pH of lower than 8.0-9.5, which, according to Akai, would affect the accuracy of distinguishing bacteria from other substances such as erythocytes and reitculocytes.

Moreover, as Applicants pointed out previously, the Examiner does not correctly apply the rule of "optimization from general conditions" to conclude that the pH range recited in the claims is merely routine optimization. As stated in MPEP2144.05.II.A, optimization can only be done within prior art conditions or through routine experimentation. Here, as noted above, none of the cited art discloses a general pH range, which would cover or at least be close to, the range recited in the claims of the present application. Nor does any of the cited art disclose any reason to use a much lower range as recited in the claims of the present application. In the present Office Action, the Examiner fails to respond to this remark.

Third, as Applicants stated previously, the unexpected results of the present application further show that the present application is not obvious in view of the prior art. The specification of the present application discusses and shows extensively various benefits of using a nitrite reducing agent and a lower pH range. *See*, for example, pages 18-20, Examples 1-2 and page 15, lines 1-5. None of the cited art provides any reasonable expectation of these benefits. In the present Office Action, the Examiner again does not respond to this argument concerning unexpected result, although the Examiner is required by the

rules to do so.

Based on the foregoing, claims 20-21, 24, 26, 28-31,35-36, and 38 are not obvious over Roth in view of Akai and Yue under 35 U.S.C. § 103. Withdrawal of the rejection of these claims over Roth in view of Akai and Yue under 35 U.S.C. § 103 is respectfully requested.

II. Obviousness rejection of claim 37

The Examiner maintains the rejection of claim 37 under 35 U.S.C. § 103(a) as being unpatentable over Roth in view of Akai and Yue, as applied above to claims 20-21, 24, 26, 28-31,35-36, and 38, and further in view of Inoue (U.S. Patent No. 5,891,733).

The Examiner only relies upon Inoue's disclosure concerning the use of ethylene glycol. Inoue cannot remedy any deficiency as discussed above in connection with other cited art. Therefore, for at least the same reasons discussed above in connection with claims 20-21, 24, 26, 28-31,35-36, and 38, claim 37 is also not obvious over Roth in view of Akai, Yue, and Inoue under 35 U.S.C. § 103(a).

It is noted that like other cited art, Inoue also discloses at col. 6, lines 52-55 that the optimal dyeing pH value is at pH 5.0 to 9.0, preferably at pH 6.5 to 7.5. This further supports Applicants' statement, as noted above, that the cited art teaches away from the pH recited in the claims of the present application.

Therefore, claims 37 is not obvious over Roth in view of Akai and Yue, and further in view of Inoue under 35 U.S.C. § 103. Withdrawal of the rejection of these claims over Roth in view of Akai and Yue, and further in view of Inoue under 35 U.S.C. § 103 is respectfully requested.

III. Double Patenting Rejection

The Examiner maintains the rejection of claims 20-21, 26 and 38 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4 and 8 of Sysmex's own U.S. Patent No. 7,422,870 issued to Kawashima et al. in view of Yue discussed above. We disagree.

In the previous response, Applicants explained that neither Kawashima nor Yue discloses a step of providing a **diluent** as recited in the claims of the present application or **a step of mixing a urine sample with the diluent prior to the step of mixing the urine sample with a staining solution**. Yue discloses that beta-mercaptoethanol may be used in a **staining solution** for greater storage stability. But Yue does not disclose that beta-mercaptoethanol should be used in a diluent, which is separate from an aqueous solution until a sample is mixed with the aqueous solution and then assayed, as a nitrite reducing agent. Therefore, the cited art, taken together, fails to provide any apparent reason that a person of ordinary skill in the art would have **combined "the known elements in the fashion claimed by the patent** at issue." *See KSR International Co. v. Teleflex Inc. (KSR)*, 550 U.S.398, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007).

In response, the Examiner again argues: "Claim 20 does not recite that 'mixing a urine sample with the diluent prior to the step of mixing the urine sample and the diluent with the staining solution.' According to claim 20, the diluent comprises the polymethine dye for staining bacteria." See, e.g., pages 15-16, item 24.

As explained above in connection with the rejection of claims 20-21, 24, 26, 28-31,35-36, and 38 under 35 U.S.C. § 103(a) over Roth et al in view of Akai et al. and Yue ST, the Examiner's construction of claim 20 is incorrect. In contrast to the Examiner's conclusion, independent claims 20 and 38 clearly describe a process comprising "mixing a urine sample with the diluent prior to the step of mixing the urine sample and the diluent with the staining solution."

For at least this reasons, the nonstatutory obviousness-type double patenting rejection of claims 20-21, 26 and 38 over claims 1-4 and 8 of Kawashima et al. in view of Yue should be withdrawn.

In addition, if in response to this Amendment, this double patenting rejection remains as the only pending rejection, then Applicants may consider filing a terminal disclaimer through a supplemental

response to overcome this rejection.

Based on the foregoing, Applicants believe that the present application is now in condition of allowance. Early and favorable consideration is earnestly requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned representative at the telephone number provided below.

If any additional fees or charges are required at this time, they may be charged to our Patent and Trademark Office Deposit Account No. 03-2412.

Respectfully submitted,
COHEN PONTANI LIEBERMAN & PAVANE LLP

By /Kent H. Cheng/
Kent H. Cheng
Reg. No. 33,849
551 Fifth Avenue, Suite 1210
New York, New York 10176
(212) 687-2770

Dated: August 5, 2009